BIOTRANSFORMATION OF PHENYL- AND PYRIDYLALKANE DERIVATIVES IN RAT LIVER 9,000xg SUPERNATANT (S-9)

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Abstract-When phenylpropanes were incubated with phenobarbital-pretreated rat liver 9,000xg supernatant (S-9), oxidative hydroxylation occurred to give phenylpropanol (racemic), (1*R*, 2*S*)- and (1*R*, 2*R*)-phenylpropanediols, (2*S*)-hydroxyphenylpropanone. Incubation of pyridylethane and propane with S-9 afforded α-pyridylethanol and propanol, but those were optically inactive. During the incubation of 1-phenylpropanone, an asymmetric redox reaction simultaneously occurred to give (2*S*)-phenylpropanol, (1*R*, 2*S*)- or (1*R*, 2*R*)-phenylpropanediols and (2*R*)-hydroxyphenylpropanone. Acetylpyridines were enantioselectively reduced to afford α-pyridylethanols in high optical yields (94-98%ee). The oxidation of pyridylalkane was significantly inhibited by cytochrome P-450 inhibitor (SKF-525A), but reduction of acetylpyridines was not inhibited. Thus, cytochrome P-450 was found to be responsible for the oxidation of pyridylalkane, but not for the reduction of the ketone.

It is known that the alkyl side chain of phenylbutazone and pentobarbital was metabolized to mainly give ω-1-C hydroxylated metabolites in rat.[1] In our previous paper, we reported that phenylbutane was regio- and stereoselectively oxidized in rat liver 9,000xg supernatant (S-9) to give (1*R*)- and (3*S*)-phenylbutanols and (1*R*, 3*S*)-phenylbutanediol in high optical yields.[2] However, few studies on the chiral redox reaction of the alkyl and carbonyl group substituted on a heteroaromatic ring have been reported.

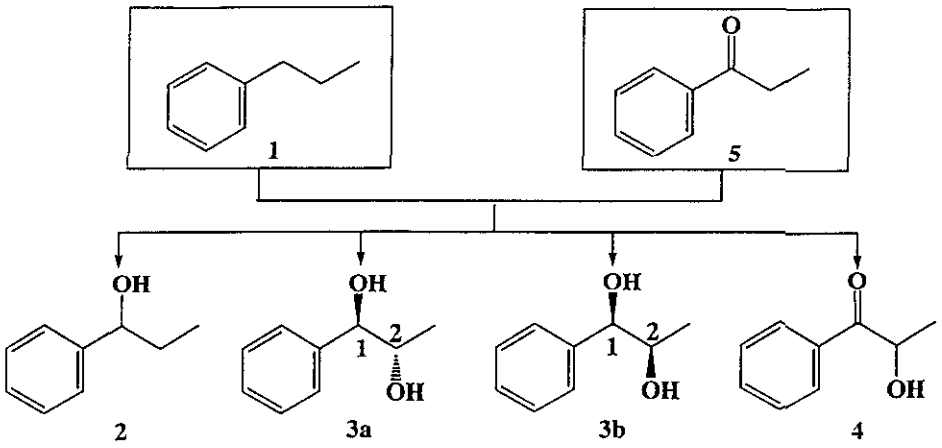
We now investigated the asymmetric redox reactions of phenyl- and pyridylalkanes or alkanones in phenobarbital-pretreated rat liver S-9.

As shown in Table 1, incubation of 1-phenylpropane (**1**) with rat liver S-9 gave four metabolites,1-phenyl-1-propanol (**2**), (1*R*, 2*S*)- and (1*R*, 2*R*)-1-phenyl-1,2-propanediols (**3a and 3b**)[3b,4] and (2*S*)-2-

Dedicated to the memory of the late Professor Yoshio Ban.

hydroxy-1-phenyl-1-propanone (4).[3b] During the metabolism, **2** was obtained as a racemic form, while **3a, 3b** and **4** were produced as optically active compounds, though the optical yield of **4** was low.

Table 1. Metabolism of Phenylpropane and Its Derivatives in Rat Liver 9,000xg Supernatant (S-9)



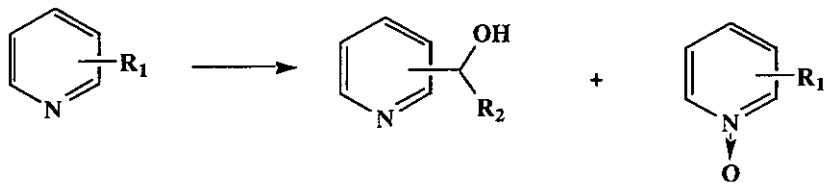
2 3a 3b 4

Product / Substrate		2	3a	3b	4
1	$[\alpha]_D$ in $CHCl_3$	0.0°	-32.6° a) (c,0.7)	-59.2° a) (c,0.1)	-32.7° a) (c,1.7)
	Optical Purity (%ee)	0	72	97	38
	Chemical Yield (%)	3	2	2	3
	Absolute Configuration	-	1*R*, 2*S*	1*R*, 2*R*	*S*
5	$[\alpha]_D$ in $CHCl_3$	-37.8° (c,9.8)	-56.5° a) (c,1.1)	-60.5° a) (c,0.5)	+5.7° a) (c,1.8)
	Optical Purity (%ee)	98	98	98	7
	Chemical Yield (%)	35	2	2	7
	Absolute Configuration	*S*	1*R*, 2*S*	1*R*, 2*R*	*R*

a) Isolated as diacetate. $[\alpha]_D$ of diacetate is shown (see ref. 3b).

When 1-phenyl-1-propanone (**5**) was incubated in rat liver S-9, an asymmetric redox reaction occurred to give (1*S*)-alcohol (**2**), (1*R*, 2*S*)- and (1*R*, 2*R*)-1, 2-diols (**3a**, **3b**), and (*R*)-ketol (**4**). In this way, from both phenylpropane (**1**) and phenylpropanone (**5**), phenylpropanol (**2**), 1, 2-diols (**3a**, **3b**) and ketol (**4**) which seemed to be the oxidized product of the diols (**3a** and **3b**) or phenylpropanone (**5**) *via* 1-phenylpropanol (**2**), were obtained as common metabolites. Optical purities of the 1, 2-diols (**3a** and **3b**) were 72-98%ee. Phenylpropanol (**2**) afforded from **1** was optically inactive, however, (1*S*)-phenylpropanol(**2**) was isolated from **5** in 98%ee. During the production of the ketol (**4**), (*S*)-ketol (**4**) was obtained from phenylpropane (**1**), while (*R*)-ketol (**4**) was produced from phenylpropanone (**5**), though the optical yield was very low.

Table 2. Metabolism of Pyridylalkane and alkanone in Rat Liver 9,000xg Supernatant (S-9)



6 7 8

No	6,8 (R_1)	7 (R_2)	Chemical yield of 7		Chemical yield of 8 (%)
			yield(%)[a]	%ee (config.)[c]	
a	2-C_2H_5	CH_3	47 (19)[b]	-	25 (15)[b]
b	3-C_2H_5	CH_3	15 (3)[b]	-	22 (14)[b]
c	4-C_2H_5	CH_3	24 (3)[b]	-	64 (42)[b]
d	2-C_3H_7	C_2H_5	47 (22)[b]	-	13 (3)[b]
e	4-C_3H_7	C_2H_5	9 (4)[b]	-	- -
f	2-$COCH_3$	CH_3	82	98 (*S*)	5
g	3-$COCH_3$	CH_3	42	98 (*S*)	1
h	4-$COCH_3$	CH_3	83	94 (*S*)	-

a) Products of 7a~e were obtained as racemic form.
b) Chemical yield of inhibited reaction of SKF-525A.
c) Optical yield (%ee) was calculated by hplc analysis.

When heteroaromatic alkanes, 2-, 3- and 4-pyridylethanes (**6a-c**) and 2- and 4-pyridylpropanes (**6d** and **6e**) were treated with rat liver S-9, α–pyridylethanols (**7a-c**)[5]and propanols (**7d** and **7e**) and *N*-oxides (**8a-d**) were produced. However, asymmetric hydroxylation did not occur, and the alcohols (**7a-e**) obtained were optically inactive. In these incubations, the alkyl groups in 2-pyridylethane and propane (**6a** and **6d**) were oxidized in preference to *N*-oxidation, while the 3- and 4-pyridylalkanes (**6b** and **6c**) were metabolized to give *N*-oxides (**8b** and **8c**) rather than the alcohols (**7b** and **7c**). From 4-pyridylpropane (**6e**), the alcohol (**7e**) was obtained, however, the *N*-oxide (**8e**) was not isolated. During the same incubation of the 2-, 3- and 4-acetylpyridines (**6f-h**), enantioselective reduction of the carbonyl groups proceeded to give (*S*)-2-, 3- and 4-pyridylethanols (**7f-h**)[5] in 82%, 42%, and 83% yields, respectively. Biotransformation of the pyridylalkanes to pyridylalcohols was inhibited by the cytochrome P-450 inhibitor (SKF-525A),[6] showing the effect of cytochrome P-450 during this oxidation. However, the reduction of the acetyl group was not inhibited by SKF-525A, and it was found that cytochrome P-450 did not participate in the reduction of the carbonyl group (Table 2).

Thus, phenylpropane (**1**) and phenylpropanone (**5**) were biotransformed in rat liver S-9 to 1-propanol (**2**), 1, 2-propanediols (**3a** and **3b**) and α-ketol (**4**), and these metabolites (except **2** from **1**) were optically active. On the other hand, during the incubation of pyridylalkanes (**6a-e**), mono-hydroxylated alcohols (**7a-e**) were produced with *N*-oxides (**8a-d**). However, the hydroxylations were not stereoselective and the alcohols (**7a-e**) were optically inactive. The oxidation of the alkyl group or *N*-oxidation was found to be influenced by the substituted position of the alkyl group on the pyridine ring. Alkyl groups in the 2-pyridylalkanes (**6a** and **6d**) were oxidized in preference to oxidation of the pyridine ring, while during the incubation of the 3- and 4-pyridylalkanes (**6b** and **6c**), *N*-oxidation proceeded faster than oxidation of the alkyl group. During the metabolism of the acetylpyridines (**6f-h**), it was found that the enantioselective reduction occurred in preference to *N*-oxidation.

As a conclusion, asymmetric oxidative hydroxylation was observed during the incubation of phenylpropane (**1**), and phenylpropanone (**5**), however, asymmetric metabolism of the pyridylalkanes (**6a-e**) was not recognized.

EXPERIMENTAL

Chemicals. Glucose 6-phosphate, NADHP was obtained from Sigma Chemical Co. (St, Louis, MO); phenylpropane from Aldrich Chemical Co. (Milwaukee, WI); SKF-525A from Research Biochemicals Inc.(Natick, MA); and sodium phenobarbital from Wako Pure Chem. Ind. Ltd. (Osaka, Japan).

Preparation of rat liver S-9 Fraction. Wistar male rats (approximately 180-200 g) were purchased from Japan SLC (Hamamatsu, Japan). Rats pretreated with sodium phenobarbital for 3 days (80 mg/kg, a day) by intraperitoneal injection were killed by decapitation and the livers were removed and quickly perfused with ice-cold 1.15% M KCl solution and homogenized with a Teflon pestle/glass mortar. The homogenate was centrifuged at 9000 xg for 20 min at or below 4 °C. The amount of protein was determined by the method of Lowry *et al.*[7] using bovine serum albumin as a standard.

Incubation Conditions and Isolation. A mixture with a total volume of 200 ml, contained S-9 (1.0 ml, equivalent to 0.33 g of liver), $MgCl_2$ (2.4 g, 60 mmol), nicotinamide (1.9 g, 16 nmol), NADP(0.2 g, 1.3 mmol), glucose-6-phosphate (1.5 g, 25 mmol), substrate (10 mmol), and 0.1 M sodium phosphate buffer(pH 7.4) was vigorously agitated at 37 °C for 1 h [Cooper and Brodie(1955)].[8] The incubation mixture was next continuously extracted with $CHCl_3$ using a Soxlet apparatus and the extract was dried over Na_2SO_4. The solvent was removed under reduced pressure to give the residue which was purified by silica gel (Wakogel C-200, from Wako Pure Chemical Industries, Ltd.) column chromatography.

Inhibition by SKF-525A. Prior to the addition of the substrate, the supernatant (S-9)(total volume, 4.5 ml) was preincubated with SKF-525A (100 μmol) in the presence of NADPH (generating system + NADP) for 5 min.

Instruments. Optical rotations were recorded on a JASCO DIP-360 polarimeter. The optical purities (%e.e.) were calculated by Mosher's method,[9] or by hplc analysis [column: Chiralcel OD and XC (Daicel Chemical Industries), solvent: hexane-propan-2-ol (95:5), 0.5 ml/min].

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Received, 2nd August, 1995